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Submicroscopic Changes in the Periventricular White Matter of Hydrocephalic ch Mouse

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Introducution

Congenitally developed hydrocephalic mice may be an ideal experimental model for human hydrocephalus. The use of hydrocephalic mutants presents distinct advantages over experimental hydrocephalic mice induced by vitamin deficiency, viruses, obstructing agents, etc. In hydrocephlic mutants, the specific type of hydrocephalus is predictable and morphological changs are uniform. They present favorable system for systematic study of anatomico-pathological aspects of congenital hydrocephalus.

The purpose of this paper is mainly to difine chronological changes of fine structure of the periventricular white matter of congenital hydrocephalic mice.

Experimental animals

The recessive mutant gene congenital hydrocephalus (ch) was discovered by GRÜNEBERG[4),5)] in 1941. He observed it in a stock which originated from the outcross of a single albino flexed-tailed male imported from the U. S. A. to females of the pure line Strong CBA.

In ch strain, inheritance of hydrocephalus is through an autosomal recessive gene and homozygotes develop hydrocephalus in utero and die immediately after birth. They are very uniform in appearance, with bulging forehead and with twin protuberances corresponding to the cerebral hemispheres and filled with hemorrhagic fluid. Development of cartilage is severely affected, biginning at the mesenchymal stage. The hydrocephalus is said to be due to an abnormal shortening of the base of the skull.

The gene, which is located in Linkage Group 14th, is maintained by sib matings of heterozygotes carrying the closely liked marker satin (sa) (Fig. 1). Heterozygotes (ch/sa) have dull coat over the abdomen and homozygotes (sa/sa) have shiny coat (Fig. 2). Because

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XIV $\dfrac{ch\ \ +}{+\ \ sa} \times \dfrac{ch\ \ +}{+\ \ sa}$

Genotype	$\dfrac{ch\ \ +}{ch\ \ +}$	$\dfrac{ch\ \ +}{+\ \ sa}$	$\dfrac{+\ \ sa}{+\ \ sa}$
Theoretical ratio	1/4	1/2	1/4
Phenotype	Hydro-cephalics	Dull coat (Breeder)	Shiny coat (Discarded)

Fig. 1. Inheritance of *ch* hydrocephalus.

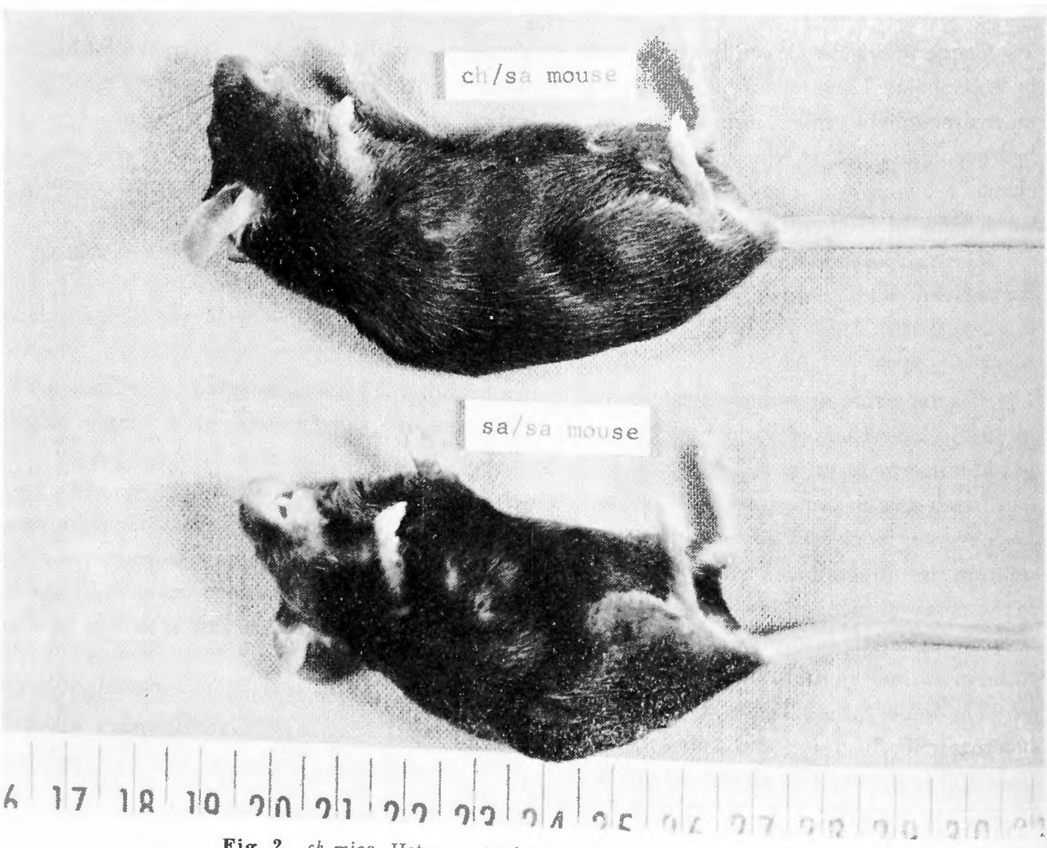


Fig. 2. *ch* mice. Heterzygote (ch/sa) has a dull coat over the abdomen and homozygote (sa/sa) has a shiny coat.

the recessive mutant gene ch causes severe embryonic hydrocephalus and the ch mice die at birth, it is necessary to study this strain in the prenatal period. The gestation period is usually 18–19 days.

Fig. 3 shows about 18-day-old normal and hydrocephalic embryos. The hydrocephalic embryo has a characteristic appearance of the head ; a steeply bulging forehead consisting of bilateral protuberances, which correspond to the cerebral hemispheres. Unlike the normal embryo, the eyes of the hydrocephalic are widely open. The nose is considerably shortened, and the general appearance of the face is distinctly pug-like.

The mechanism of the development of hydrocephalus in the ch embryo was first described by GRÜNEBERG[4]. The dorsal bulging of the telencephalon is a mechanical consequence of the shortening of the anterior part of the cartilaginous basis of the skull. The initial widening of the lateral vantricles, which begins at about 12 days gestation, is brought about not by an increased production of CSF, but by the deformation of the hemispheres, when these are forced up dorsally by the lack of co-ordination between growth of the brain and growth of the base of the skull. In later stages, the ventricles are blown up by hydrostatic pressure. The dilatation is confined to the lateral ventricles ; the other ventricular system is not dilated. Apparently the stress set up by the expanding cerebral hemispheres against the elastic counterpressure of the skull tends to compress the remainder of the brain. Though the remainder of the ventricular system is narrowed down, its open community is nowhere interrupted. However,

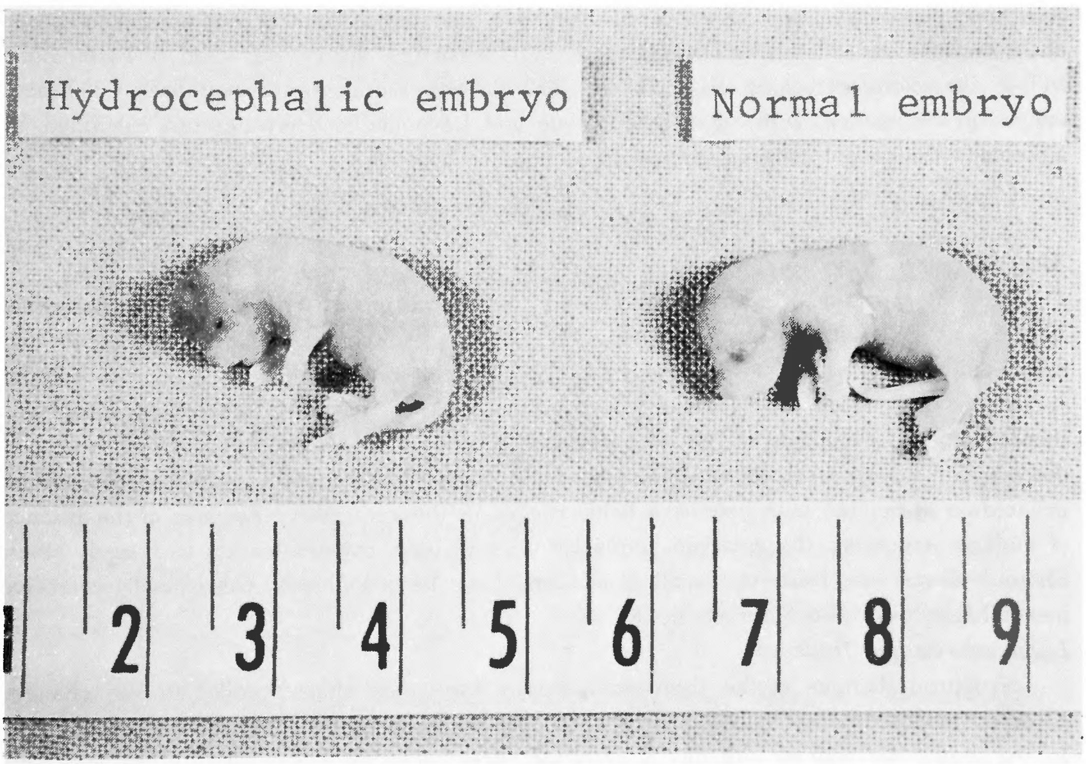


Fig. 3. About 18 gestational days normal and hydrocephalic embryos. The hydrocephalic embryo has a characteristic appearance of the head; a steeply bulging forehead consisting of bilateral protuberances, which correspond to the cerebral hemispheres.

an obstruction eventually develops in the neighbourhood of the foramen of Magendie. It is little doube that the absence of a proper foramen of Magendie and the virtual absence of pia-arachnoid space will greatly impede the drainage of the CSF and the cerebral hemispheres are blown up enormously by hydrostatic pressure. The hemorrhages usually take place near the midline. They are obviously due to rupturing of the blood sinuses which must be under considerable strain.

Methods

On the later day of gestation ch pregnant mice were anesthetized with intraperitoneal administration of 0.4ml of 4% chloral hydrate. The abdomen was opend and caesarean section was made. After fetuses were grossly examined, they were decapitated immediately. Their whole heads were divided into two groups. For light microscopic study the whole heads were fixed with Newcommer's* and Bouin's solution for 2 days or longer and embeded in paraffin, and 7μ thick serial sections were stained mainly with hematoxylin and eosin.

For electron microscopic study, top of the head was rapidly cut immediately after the fetus was delivered by caesarean section. Small pieses of top of the head were fixed in 5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) for 3 hours at 0°C and rinsed in 7.5% sucrose in 0.1M cacodylate buffer (pH 7.4) 3 times for 10 minutes each. The specimens were then postfixed in 1% buffered osmium tetraoxide for 2 hours at 0°C. The tissue was dehydrated through graded alcohols and propylene oxide, and embedded in Epon and Araldite. Dorsolateral roof of the lateral ventricle, selected by light microscopic examination of 1μ sections stained with methylene blue, was sectioned at 500–800 Å on a Porter-Blum MT-2 ultramicrotome using glass knives. The ultrathin sections were mounted on 300 mesh copper grids, stained with 3% uranyl acetate and Raynolds' lead citrate, and examined by a Siemens Elmiskop I electron microscope.

Results

Macroscopic findings

After 12 gestational days the lateral ventricles of the normal embryo became somewhat smaller and the pallium became thicker. Fig. 4 shows a coronal section of normal and hydrocephalic embryos of 17–18 days old gestation. In hydrocephalic embryo, the size of the ventricle increased and the thickness of the pallium decreased as the degree of hydrocephalus increased. Gross changes in the advanced hydrocephalic embryos were extreme atrophy of the cerebral mantle with thinning of the white matter and frequent rupture of the septum pellucidum associated with extensive hemorrhages in the ventricles. Because of the absence of midline structures, the enlarged ventricles were in open communication with each other. Choroid plexus was relatively small and seemed to be artophied. Occasionally extensive hemorrhages were also observed in the orbit.

Light microscopic findings

Structural changes at the light microscopic level were almost similar to the changes observed in experimental hydrocephalus which had been reported by other investigators[2),6),11)]. In the ventricular wall of 12th day of gestation, three layers, ependymal, mantle and marginal,

* Isopropanol 6 parts, Propionic acid 3 parts, Acetone 1 part, Dioxane 1 part, Light petroleum (ligrine) 1 part.

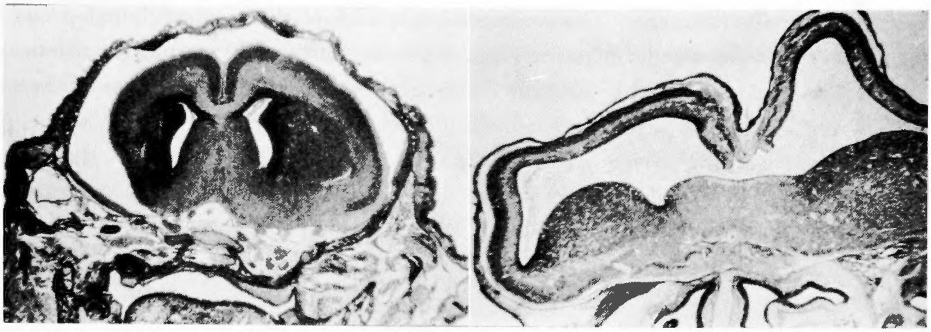


Fig. 4. Coronal section of a whole head of 17-18 gestational days embryo. Fixed in Bouin's solution (normal) and in Newcommer's solution (hydrocephalic) and embeded in paraffin. H. & E., 35X.
In the normal embryo, the lateral ventricles are slit-like narrow. (The space between skull bone and brain is an artefact)
In the very advanced hydrocephalic embryo, the lateral ventricles are markedly dilated and the pallium is thinned out consiberably; the lateral ventricles are in widely open communication with each other. (The configuration of hemispheres is an artefact)

were observed. The ependymal lining was a thick stratified layer. The ependymal lining of embryo of later gestational days was not single layer yet. The ependyma of hydrocephalic embryo was moderately flattened and the number of small capillaries increased in the subependymal layer. Size of the capillaries in hydrocephalic embryos was generally larger than in normal embryos. The ependyma appeared relatively intact but the subependymal layer showed spongy appearance (Fig. 5). On rare occasion, the ventricle was filled with

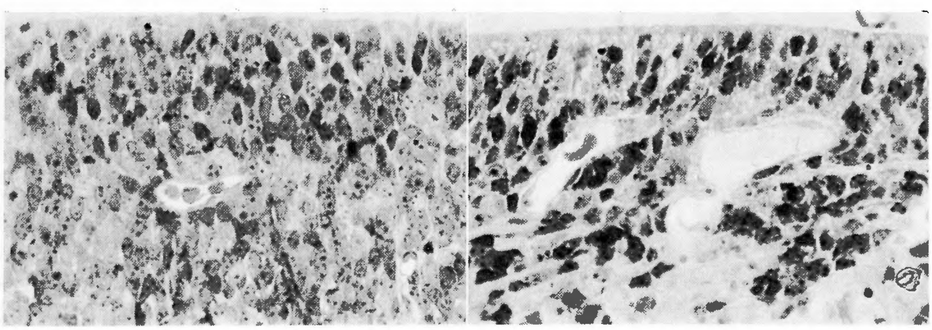
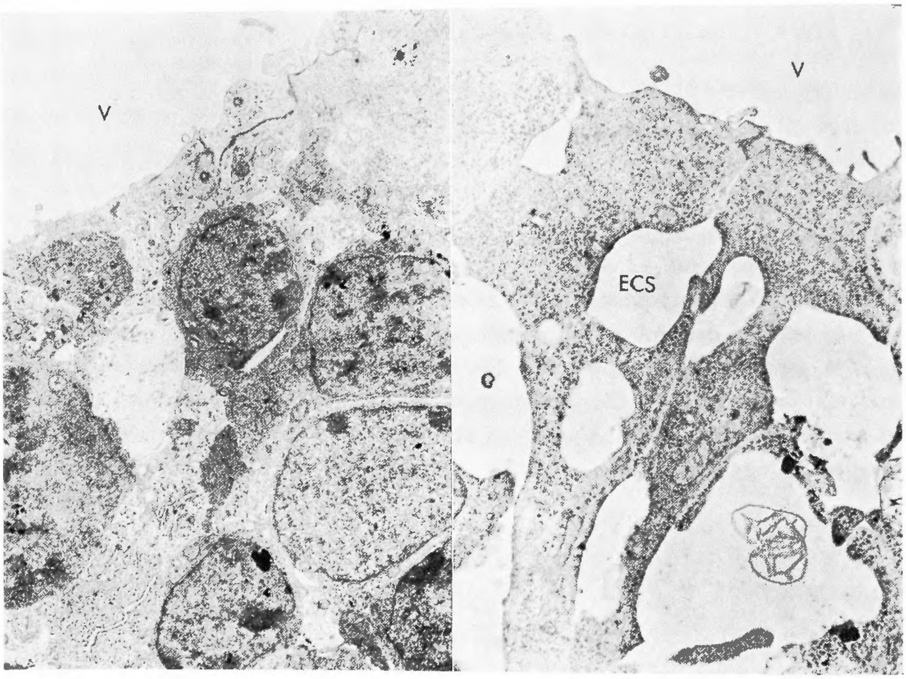


Fig. 5. The periventricular white matter of 17 gestational days embryo. Methylene blue 400X. In the normal embryo, the ependymal lining is a stratified layer. In the hydrocephalic embryo, the ependyma appears relatively intact but the subependymal layer shows spongy appearance. The number of small capillaries is seemed to be increased.

hemorrhagic fluid and severe ependymal destrution was noted. Sometimes hemorrhagic fluid was observed within both ventricle and subependymal layer.

Electron microscopic findings

The most prominent finings observed in the periventricular tissue of hydrocephalic ch mouse embryos at the electron microscopic level was an increase of the extracellular spaces in the subependymal structures. The extracellular spaces of the subependymal white matter in normal embryos were small and somewhat larger in early embryogenesis. Measurement of the extracellular space of the electron micrographs of normal embryos showed that about 8 % at the age of 12 gestation gradually decreased its value down to about 6 % at newborn. By contrast, the space in hydrocephalic embryos increased as the degree of hydrocephalus increased. The value of about 11% at 12 gestational days increased up to about 27% at 18 gestational days. The ependymal lining of hydrocephalic embryos was relatively intact and the intercellular junctions communicated with the enlarged extracellular spaces (Fig. 6).



Normal Hydrocephalic

Fig. 6. Electron micrographs of the dorsolateral part of the lateral ventricle of 17 gestational days *ch* mouse embryo.
Immersed in glutaraldehyde and postfixed with osmium tetraoxide. In the normal embryo, the nucleus of ependymal cells is generally round to oval. The extracellular spaces of the periventricular white matter are small. X 2,600.
In the hydrocephalic embryo, the ependymal lining is relatively intact, but the extracellular spaces are apparently widened and the intercellular junctions communicate with enlarged extracellular spaces. X 3,800.
(V: lateral ventricle, ECS : extracellular space)

Basically no marked difference between normal and hydrocephalic embroys in nucleus and cytoplasm of the ependymal cells was found. In the early stage of embryogenesis, the ependymal lining was multilayered and the apex of the cells bordering the ventricle contained more cytoplasm. The cells beneath the apical cell had scant cytoplasm. Their nuclei were generally round to oval and the cell processes were not long yet. Around 14 days gestation cytoplasm protruded into the ventricular lumen but no cilia were found. The lateral cell junctions were relatively straight. The cells became more differentiated. The shape of nuclei was somewhat irregular. The cytoplasm had many ribosomes. Immature cilia first appeared at later embryogenesis after about 17 days of gestation.

Discussion

Studies on ch mice have been reported only by GRÜNEBERG AND GREEN. GRÜNEBERG[4] discribed genetics of ch mice. Developement of congenital hydrocephalus was studied by inspection of the external features of a series of embryos and newborns. Green[3] exmained macroscopic and light microscopic change of congenital hydrocephalus. There are few investigations of the embyonic periventricular white matter with the electron microscope.

The ependyma lining the ventricular system differs morphologically from one area to another. In hydrocephlic mice, the structural changes are much more severe in the roof of the lateral ventricles than the other parts of the ventricular system. In order to compare the periventricular white matter of normal and hydrocephalic embryos in various gestational ages, the tissue was selected from the same areas lining the dorsolateral part of the lateral ventricles, and was studied microscopically and submicroscopically.

Difficulty in preparation and cutting of whole head of the embryo for electron microscopic study was encountered. Even if plastic penetrates well into the brain tissues and ventricles during embedding, plastic whithin the tissues was too soft to cut. Inadequate plastic polymerization was supposed to be due to incomplete dehydration of the brain tissues. Therefore, instead of the whole head, top of the head and its pieces were used in this study.

In hydrocephalus morphological changes mainly involve the white matter[19]. In acute experimental hydrocephalus, excessive fluid accumulates in the periventricular white matter, apparently due to the change in ependymal permeability secondary to increased intraventricular pressure. The most prominent feature of the periventricular white matter of hydrocephlic ch mice at the electron microscopic level is an increase of the extracellular spaces in the subependymal structures. BRIGHTMAN AED REESE[1] have demonstrated the presence of extracellular spaces communicating with the CSF compartment with the electron microscope in the normal animal brains. In the studies of experimental hydrocephalus[7],[8],[18], as the degree of hydrocephalus increases, the extracellular space in the periventricular subependmal structures increases. The periventricular white matter becomes spongy and edematous. It is well known that the ependyma is relatively permeable to variety of substances injected into the CSF[13]. In hydrocephalus, this permeability is much more greatly increased as result of high pressure whithin the ventricles and the structural changes in the ependyma such as stretching and disruption. In hydrocephalic ch mice, the ependymal lining is relatively intact even at the later stage of gestation and the intercellular tight junctions are usually preserved. But the extracellular spaces are apparently widened and the intercellular junctions communicate with enlarged extracellular spaces. Such submicroscopic changes suggest that permeability of the ependyma is altered in hydrocephalus, resulting in greater penetrance of fluid into the subependymal white matter, and certain transependymal pathway of CSF circulation is established in advanced hydrocephalus. It is conceivable that the extracellular spaces in the white matter close to the ventricular lumen enlarge with hydrostatic pressure in hydrocepalus and these spaces may be the channels through which the CSF flows to be absorbed by the capillaries within the white matter[14],[15],[16]. Although the submicroscopic changes in the capillaries within the periventricular white matter were not investigated in this study, light microscopic study shows that the number of small capillaries in hydrocephalus increased in the subependymal layer and the size of capillaries was generally larger than in normal. SAHAR et al.[17] have also observed numerous blood vessels in the white matter close to the ventricle. The increase of blood vessels in number may be due to the differential atrophy of the cerebral mantle proper, or may be represent that preexisting blood vessels have become larger due to active or passive congestion. From location, number and size of the vessels, their increase might be related to enchancing the absorption of excessive CSF[9] SAHAR et al.[17] also suggested the development of an alternative transventricular route for CSF absorption in spontaneous canine hydrocephalus. According to their results, the process of transventricular absorption actually took place within the layers closest to the ventricular cavity. LUX et al.[10] also showed that when intraventricular pressure was increased by perfusion of the cerebral ventricles in cats with experimental chronic hydrocephalus, there was an increase in the water content and inulin space within the first 600μ of brain adjacent to the ventricle. It was concluded that the increased water and carbon 14-labeled inulin content measured in the periventricular white matter was derived from the CSF compartment during transventricular absorption.

MILHORAT and HAMMOCK's recent study[12] on ventricular size and configuration in human hydrocephalus by isotope ventriculography using I^{131} serum albumin suggests some correlation with morphological changes in hydrocephalus. Isotope ventriculograms demonstrated a "double density" pattern. The inner, more dense zone of the uptake conforms to outline of the ventricular system. The outer, less dense zone extended around the ventricles apparently represents radioisotope in the brain parenchyma. The outer zone may correspond to the uptake in the enlarged extracellular spaces in the periventricular tissue.

Summary

The periventricular white matter of ch hydrocephalic mouse embryos was studied microscopically and submicroscopically.

In the hydrocephalic embryos, the most prominent change in the white matter close to the lateral ventricle was an increase of the extracellular spaces. The ependymal lining was relatively intact even at the later stage and the intercellular tight junctions were usually preserved. The enlarged extracellular spaces had a communication with the intercellular junctions. As the degree of hydrocephalus increased, the extracellular spaces in the periventricular subependymal white matter increased. The number of the small capillaries also increased in the subependymal layers. Such morphological changes suggest that ependymal and vascular permeability is altered and certain transependymal pathway of CSF circulation is established in the advanced hydrocephalus.

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和文抄録

先天性水頭症 (ch) マウスの脳室近傍白質の微細変化

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先天性水頭症マウス胎児の側脳室近傍白質の経時的微細変化を，光学顕微鏡ならびに電子顕微鏡レベルで追究した．

先天性水頭症マウスにおいても，これまでに報告された実験的水頭症におけると同様，側脳室近傍白質中での細胞外腔の著明な拡大を認めた。多くの場合，脳室壁上衣細胞ならびに上衣細胞間の密接合部は比較的その形態が保たれており，拡大した細胞外胞が上衣細胞接合部を通じて脳室と交通していた．脳室近傍白質中の細胞外腔は，水頭症の程度が増強するにつれ拡大していき，白質中の毛細血管の数，大きさも増していくことが観察された．

このような形態学的変化は，水頭症における上衣細胞ならびに血管透過性の変化にもとづくもので，高度の水頭症において，脳脊髄液循環の経上衣細胞側副路が形成されていることを示唆する．